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S/N 08/881,393

PATENT 08/01/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	FODSTAD ET AL.	Examiner:	C. CHIN
Serial No.:	08/881,393	Group Art Unit:	1641
Filed:	JUNE 24, 1997	Docket No.:	7885.33USD1
Title:	METHOD FOR DETECTION OF SPECIFIC TARGET CELLS IN SPECIALIZED OR MIXED CELL POPULATION AND SOLUTIONS CONTAINING MIXED CELL POPULATIONS		

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited in the United States Postal Service, as first class mail, with sufficient postage, in an envelope addressed to: BOX AF, Assistant Commissioner for Patents, Washington, D.C. 20231 on . May 22 2000

By: John J. Fresenj
Name: John J. Fresenj

SUPPLEMENTAL RESPONSE

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In further response to the final Office Action mailed February 14, 2000 and our response dated May 15, 2000, submitted herewith are copies of the following two abstracts for the Examiner's review and consideration. Applicants believe these cited references show examples of the presently claimed method. Reexamination and reconsideration of the application in light of these references is respectfully requested.

Fodstad, et al., "#172 Improved immunomagnetic method for detection and characterization of cancer cells in blood and bone marrow", *Proceedings of the American Association of Cancer Research*, Vol. 38, pp. 26 (March 1997).

Fodstad, et al., "#1463 New Immunobend techniques for sensitive detection of malignant cells in blood and bone marrow", *Proceedings of the American Association for Cancer Research"*, Vol. 37, pp. 26.

If the Examiner believes a telephone conference would be helpful in resolving any issues concerning this communication, the Examiner is invited to telephone the undersigned at the below listed telephone number.

Respectfully submitted,

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Dated: May 22, 2000

John J. Giesens
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PBSC mobilization with TAC + G is superior to that with FAC + G allowing optimal collection of PBSC for hematopoietic rescue after high-dose chemotherapy.

#168 Mobilization of peripheral blood stem cells (PBSC) after primary intense chemotherapy and granulocyte-colony stimulating factor (G-CSF) in breast cancer. Charrier, S., Communal, Y., Curé, H., Chollet, P., Portefaix, G., Ferrière, J.P., Bignon, Y.J., Bétail, G., Plagne, R., and Chassagne, J. *Centre Jean Perrin and INSERM CRI 9402, BP 392 - 63011 Clermont-Ferrand, France*

The aim of this study was to ascertain PBSC mobilization after an effective regimen in breast cancer (30% of pathological complete response (ASCO 95, 14, 218)) and look for a sufficient PBSC collection for reinjection. Between January 1995 and June 1996, 15 breast cancer patients were treated by TNCF (THP-doxorubicin, vinorelbine, cyclophosphamide, fluorouracil for 4 days, every 21 days) and received G-CSF support (filgrastim 5 μg/kg D5 to D14) to reduce aplasia. During the first intercourse, leukocytes, mononuclear cells (MNC) count, Colony Forming Unit-Granocyte Macrophage (CFU-GM) assay, CD34$^+$ cells count through 5 anti-CD34 antibodies and DNA synthesizing MNC count were performed daily, between D12 and D17 post-chemotherapy (81 samples). The results showed an homogenous profile for PBSC mobilization and haematological recovery. The leukocyte count reached 3,200 (1,100–6,300)/μl of blood on D12 and 25,847 (2,500–63,100)/μl of blood on D15, which was the day of leukocyte optimal count. The three methods used for PBSC evaluation were correlated ($p<0.01$) and showed a good peripheral mobilization with an optimal PBSC recruitment on the last day of G-CSF administration (D14):

D14	CFU-GM	CD34$^+$ cells	Cycling MNC
Mean (range)	86 (1–328)	258 (11–1,341)	203 (9–554)

In conclusion, these three methods showed a good mobilization with this regimen at the last day of G-CSF administration; furthermore, the optimal count of leukocytes was found the following day.

#169 Randomized trial of sequential Filgrastim (G-CSF) and Molgramostim (GM-CSF) versus Filgrastim alone after high-dose chemotherapy (HDCT) with peripheral blood progenitor cell (PBPC) support. Recchia, F., De Filippis, S., Fincato, G., and Rea, S. *Oncology Unit, Avezzano, Italy, Sandoz, Milano, Italy, Surgical Oncology University of L'Aquila, Italy, C.R.O.F.I., Monterotondo, Italy*

G-CSF and GM-CSF have synergistic and differential effects on hematopoietic stem cells. G-CSF can act on very primitive stem cells as well as later hematopoietic progenitors of granulocytic lineage. GM-CSF action, restricted to the terminally differentiating cell of all lineages, can have pleyotropic effects if administered when hematopoietic progenitors resume their growth. From 1-94 to 11-96 the study was conducted to evaluate the role of the sequential administration of G-CSF and GM-CSF compared to G-CSF alone after HDCT and PBPC. HDCT was given day (D)1 to D3, PBPC were infused on D5 and growth factors were administered, subcutaneously, 5 mg/kg. Randomization was as follows: arm A, 10 patients (PTS), G-CSF D4 to D17; arm B, 10 PTS, G-CSF D4 to D10, GM-CSF D11 to D17. PTS characteristics were homogeneous in both arms for age, performance status and # of CD34+ infused. Median number, per patient, of days with:

Arm	PLT <20,000	WBC <1,000	Fever	Mucositis	Hospitalization
A	3	5	6	25	20
B	0.5 ($p<0.05$)	6 ($p>0.05$)	0 ($p<0.05$)	0 ($p<0.05$)	22 ($p>0.05$)

(Mann-Whitney sum rank test). After a median follow-up of 14 months, 13 PTS were disease free and 16 were alive. These preliminary data show an improved tolerance of HDCT with PBPC and sequential G-CSF and GM-CSF.

#170 CD34+ leukocyte count in peripheral blood under steady state conditions predicts neutropenia after administration of CPT-11 and cisplatin for primary lung cancer. Egusa, Y., Fujiwara, Y., Isobe, T., and Yamakido, M. *2nd Dept. of Internal Medicine, Hiroshima Univ. School of Medicine, Hiroshima 734, Japan*

Although CPT-11 has been shown to be active against lung cancer, its main side effect of neutropenia is often troublesome. At present, it is difficult to predict neutropenia before administration of anti-cancer agents. On the other hand, leukocyte surface antigen CD34 is generally recognized to be an indicator of the quantity of peripheral blood stem cells (PBSCs). Therefore, we examined the correlation between PBSCs count and neutropenia after administration of CPT-11 and cisplatin to patients with lung cancer. Twelve patients received CPT-11 and cisplatin at the normal dose, total twenty courses were estimated. Obtained peripheral blood samples were assayed for PBSC by flow-cytometry using CD34 antibody twice a week. Retrospectively, we placed patients who developed neutropenia in Group A (n=6) and those who did not in Group B (n=14). We found a significant difference between Group A (943.8±379.6) and Group B (2643.8±1280.3), ($p<0.01$) in the CD34+ leukocyte count in peripheral blood under steady state conditions (Steady CD34+ in PB). These results suggest that neutropenia can be predicted by estimating Steady CD34+ in PB before administering CPT-11 and cisplatin to patients with primary lung cancer.

#171 The chemosensitivity test is useful in evaluating the appropriate cancer chemotherapy against advanced gastric cancers. Fujita, K., Kubota, T., Matsuzaki, S.W., Otani, Y., and Kitajima, M. *Department of Surgery, School of Medicine, Keio University, Tokyo 160, Japan*

One hundred fifteen patients with advanced gastric cancer were enrolled in the study, in which the chemosensitivity test with MTT endpoint was used to predict the chemosensitivity of the individual patient. Agents used are mitomycin C (MMC), doxorubicin (DXR), 5-fluorouracil (5-FU), and cisplatin (DDP), of which cutoff concentrations are 10, 10, 50 and 25 μg/ml. Efficacy rates of the drugs were 10.4% for MMC, 5.2% for DXR, 6.1% for 5-FU, and 12.2% for DDP, which were similar to the efficacy rate of each drug reported clinically. Although there were no significant correlations between the chemosensitivity and the background factors including sex, age and pathological stage, it was observed that differentiated carcinomas had higher sensitivity to the agents (28.8%) comparing with undifferentiated carcinomas (9.5%) ($p<0.05$). Measurable lesions remained in 17 patients after the operation and the clinical effects of the drugs were compared with the sensitivity of the specimens. They included 3 true positive, 12 true negative and 2 false positive cases, resulting in 88% accuracy with 100% sensitivity, 86% specificity, 60% true positive and 100% true negative rate. The MTT assay seemed to be useful in evaluating the appropriate cancer chemotherapy against advanced gastric cancers.

#172 Improved immunomagnetic method for detection and characterization of cancer cells in blood and bone marrow. Fodstad, Ø., Øverli, G.E.T., Forus, A., Rye, P.D., Beiske, K., Aamdal, S., and Hellodt, H.K. *The Norwegian Radium Hospital, 0310, Oslo, Norway*

The presence of circulating tumor cells in patients without overt metastases may indicate an increased risk of relapse. Sensitive and specific detection of such cells can therefore be of prognostic value, and the results may aid in choosing therapeutic alternative and as a surrogate marker for monitoring response to treatment. We have developed a rapid and sensitive immunomagnetic procedure for identification of tumor cells disseminated to blood and bone marrow in patients with breast, colorectal, prostate, and ovarian carcinomas, and with malignant melanoma or osteosarcoma[1,2]. A detection level of 2–5 cancer cells per 10^7 mononuclear cells has been achieved, and a close correlation was observed between changes in the presence of contaminating melanoma cells and response to chemotherapy. The method allows further characterization of the selected malignant cells.Using FISH, amplification of the ERBB2 gene in breast cancer cells could be demonstrated in model experiments and in a clinical specimen. Further methodological development shows promise for more detailed characterization of the malignant potential of the contaminating tumor cells.

1. Fodstad et al., Proc Am Ass Ca Res. 37: 214, 1996

2. Fodstad, Ø. In: Clinical significance and detection of tumor cells in blood and bone marrow (Riethmuller et al., eds) pp. 59–66. Pharmacia &Upjohn, Lund, 1996

#173 Determination of retinoic acid receptor b (RARb) expression by RT-PCR ELISA: Application to head and neck tumors (HNT). Castillo, L., Pierrefite, V., Santini, J., Milano, G., and Schneider, M. *Hôpital Pasteur [L.C., J.S.], Centre A. Lacassagne, Nice, France [V.P., G.M., M.S.]*

RARb expression is decreased during the cancerous process and restored by retinoid treatment. The main purpose of this study was to develop and clinically validate a sensitive assay for measuring RARb expression in small tumor samples (5–20 mg). The method was based on the principle of RT PCR ELISA which consists in generating digoxigenin-labeled PCR products hybridized to a specific biotinylated probe complementary to the inner part of the amplification product. The generated hybrids are immobilized on a streptavidin coated microtitration plate. Detection was performed with an anti-digoxigenin peroxidase conjugate. This study was applied to the exploration of 20 laryngeal tumors (T) and 20 normal mucosa from non cancer patients (NT). RARb/b2 microglobulin ratios were respectively for T and NT(median, extremes): 450, 115-1310 and 926, 349-2155 ($p = 0.001$), thus confirming the loss of RARb expression in HNT. This simple fast and sensitive method (detection limit at 50 pg of PCR product) could be used for testing RARb as a biomarker of the carcinogenic process and helping select new retinoids in chemoprevention.

IMMUNOLOGY/PRECLINICAL AND CLINICAL BIOLOGICAL THERAPY 1: Antibodies, Immunoconjugates, Tumor Antigens

#174 Antitumor effects of L49-sFv-β-lactamase in combination with a cephalosporin mustard prodrug. Kerr, D.E., Vrudhula, V.M., Siemers, N., and Senter, P.D. *Bristol-Myers Squibb, Seattle, WA 98121*

The role of exogenous estrogen therapy in the development of breast cancer remains controversial. Data suggest that postmenopausal (POST) women who have received estrogen therapy prior to breast cancer diagnosis may have a more favorable prognosis. This improved prognosis may reflect earlier diagnosis or the development of tumor estrogen receptor (ER) positivity. We have analyzed the use of exogenous estrogens in 325 breast cancer patients (pts) (128 premenopausal (PRE), 197 POST) in relationship to ER content of primary breast cancers. Age, menopausal status, hormone usage prior to cancer diagnosis, and ER and progesterone receptor content were recorded for all pts. Hormone usage (contraceptive and fertility drugs (3 pts) in PRE pts and/or conjugated estrogens in POST pts) was recorded as never (NEVER) used or some (SOME) use (past usage or current usage (within 6 months of diagnosis).

	NEVER			SOME		
	n	ER*	PR*	n	ER*	PR*
TOTAL	102	51.0 ± 1**	92.9 ± 29	223	79.2 ± 11**	107.9 ± 13
PRE	53	38.7 ± 7	130.1 ± 54	75	48.6 ± 8	116.2 ± 24
POST	49	64.6 ± 15'	53.4 ± 16	148	94.5 ± 16'	103.8 ± 15

* mean ± SEM. ** $p = 0.04$, ' $p = 0.057$

Pts who received estrogen therapy developed breast cancers with significantly higher ER content than pts who had never received estrogens ($P = 0.04$). The higher ER level may explain the improved prognosis of hormone-user pts.

#1461 Tuesday, April 23, 1996, 1:00–5:00, Poster Section 16

p53 Protein overexpression is associated with premalignant cytologic changes in the breast. Kamel, S.*, Zeiger, S., Zalles, C., McKittrick, R., Kimler, B.F., Simon, C. and Fabian, C.J. *University of Kansas Medical Center, Kansas City, KS 66160.*

We hypothesized that morphologic and molecular changes may exist in high-risk women prior to breast cancer development. Although p53 protein overexpression and/or gene mutation can be detected in ≥50% of breast cancers, it is not clear how early in breast tumorigenesis these changes occur. We studied p53 protein expression in breast fine needle aspirates (FNA) of a high risk cohort, using immunocytochemistry. In addition to cytologic evaluation, aspirates were also tested for DNA aneuploidy and overexpressed ER, EGFR and Her-2/*neu*, PAb240 antibody, which recognizes an epitope generally associated with mutant p53 protein, was used. High-risk women (n = 213) were those with breast cancer in a 1st degree relative (73%), prior biopsy indicating premalignant breast disease (26%, including atypical hyperplasia or carcinoma in situ), prior breast cancer in the contralateral breast (13%), or some multiple thereof (11%). Thirty low-risk control women were also aspirated. p53 protein was overexpressed in 28% of high-risk versus 3% of low-risk subjects. There were no significant differences between high-risk subcategories in prevalence of p53 overexpression. There was a significant increase in the prevalence of p53 overexpression with increased cytologic abnormality (10% normal cytology, 32% epithelial hyperplasia and 50% hyperplasia with atypia; $p < 0.003$). At a median follow-up of 2 years, 9 of 213 high-risk women have developed in situ (n = 5) or invasive (n = 3) cancer. p53 overexpression was associated with hyperplasia with atypia ($p = 0.002$), which in turn was strongly predictive of later cancer development ($p = 0.0006$). It did not correlate with any of the other biomarker abnormalities, except Her-2/*neu* ($p = 0.0001$). Our results suggest that p53 overexpression can be demonstrated in hyperplastic breast tissue with and without atypia and is a potential risk biomarker in a population of women at high risk for breast cancer.

#1462 Monday, April 22, 1996, 02:55–03:10, Room 10

Serum concentrations of soluble TNF-α receptors and β2-Microglobulin in multiple myeloma patients after immuno-chemotherapy. Dosser, L., Sakalova*, A., Zavadova, E., Holomanova, D*., and Mohr, T. *Institute of Tumorbiology/Cancer Research, Applied and Experimental Oncology Department, University of Vienna, Austria. *Clinic of Hematology, University of Bratislava, Slovakia.*

Soluble forms of the two receptors for tumor necrosis factor (TNF) are present in human sera. Their concentrations increase greatly with the progress of certain diseases. We determined soluble tumor necrosis factor receptors (sTNF-R p55 and sTNF-R p75) and β2-Microglobulin (β2M) concentrations in the sera of 197 patients with multiple myeloma stage I-III: before therapy i), after chemotherapy ii) (MOCCA/VMCP) or after immuno-chemotherapy iii) (WOBE-MUGOS a polyenzym preparation - MOCCA/VMCP). A group of 67 age matched healthy volunteers served as control. The concentration of sTNF-Rs and β2M were significantly ($p < 0.05$) higher in the sera of MM patients. A stage dependency as well as a correlation between the concentration of sTNF-Rs and β2M was observed. The concentration of sTNF-R and β2M in sera of patients after immuno-chemotherapy was lower compared to that of patients treated with chemotherapy alone.

#1463 Monday, April 22, 1996, 03:25–03:40, Room 10

New immunobead techniques for sensitive detection of malignant cells in blood and bone marrow. Ø. Fodstad, H.K. Hoifødt, P.D. Rye, G.E. Trunes and K. Beiske, *Inst. for Cancer Research, The Norwegian Radium Hospital.*

A rapid immunomagnetic method for detection of tumor cells in body fluids has been developed. Selected monoclonal antibodies, with no cross-reactivity to normal cells in blood (PBL) and bone marrow (BM), were used together with Dynabeads for magnetic isolation of target cells. Unspecific binding of beads to cells was avoided by optimizing the incubation procedures, permitting quantification of the selected tumor cells by counting the number of cells with rosettes of magnetic beads under light microscopy. In model experiments, a sensitivity of 2–4 tumor cells in $>1 \times 10^7$ MNCs was achieved, a sensitivity superior to that obtained with immunocytochemistry. Importantly, the immunobead technique can be performed equally on samples of PBL and BM from cancer patients, and the whole procedure can be completed within less than 2 hrs. The method can also be used on MNC fractions that have been frozen. Studies on material from patients with breast and colorectal carcinoma and with malignant melanoma have shown similar sensitivity and specificity to that found in the model experiments. These results, in addition to the speed and simplicity of the new procedure makes it particularly attractive for detection of micrometastatic disease, and monitoring response to therapy. The magnetic isolation also permits biological, immunological and molecular characterization of the tumor cells. This antibody-bead procedure maybe combined with a filtering process that removes unbound beads and provides feasibility for culturing tumor cells. Data obtained with this method may prove valuable for disease staging, as a prognostic indicator in selection of therapeutic approach and a marker of response to therapy.

#1464 Sunday, April 21, 1996, 8:00–12:00, Poster Section 10

New drug to lower uric acid levels in healthy volunteers. Advances on prevention and control of hyperuricemia in malignancies? Jacob, F.*, Drelon, E.**, Neefe, J.***, Lascombes, F.**, *CHU Vandoeuvre F54511, **Sanofi-Recherche Montpellier F34184, and ***Sanofi-Research Great Valley PA19355.

Introduction: two types of antihyperuricemic agents are currently used to lower serum uric acid levels, those which decrease uric acid production (allopurinol...), or those which increase uric acid excretion by the kidney (probenecid...). A third therapy should be proposed for the treatment of hyperuricemia: the administration of urate oxidase (SR 29142). A clinical trial in healthy volunteers (n = 28) was performed to assess the safety, the efficacy, and kinetics of single, then repeated, increasing doses of SR 29142.

Safety results: no serious adverse events were noted during the observation period. Two volunteers presented transient headaches. No important clinically changes were seen.

Efficacy results: following single intravenous (IV) of SR 29142, 78.4%, 84.3%, 91.9% and 96.0% decreases from baseline values in extemporaneous plasma uric acid levels were recorded in the 0.05, 0.10, 0.15, and 0.20 mg/kg groups (n = 4 per group) by 8 hours after dosing, respectively. In daily IV administrations for 5 days of SR 29142, a rapid and marked fall in uric acid levels was obtained in all groups after SR 29142 administration. The levels remained very low during the remainder of the treatment period and were similar in all the groups (on day 6: 94.9%, 96.6%, and 94.6% decreases from baseline values in extemporaneous plasma uric acid levels were seen in the 0.10, 0.15, and 0.20 mg/kg groups (n = 4) after SR 29142 administration, respectively.

Conclusion: Because of the rapidity of uricolytic action of SR 29142 and its persistent activity over a period of several days, its administration will be particularly indicated in the therapy for malignancy-associated hyperuricemia, and prophylaxis of and therapy for hyperuricemia associated with the treatment of malignancies.